application

Agilent Technologies
Innovating the HP Way

Liquid Chromatography/Mass Spectrometry | Toxicology

Determination of LSD, Iso-LSD, and 2-Oxo-3-Hydroxy-LSD in Urine Using the 1100 LC/MSD

Matthew H. Slawson, Carol L. O'Neal, Thomas C. Kupiec, and Rodger L. Foltz
Center for Human Toxicology,
University of Utah, Salt Lake City, UT

John M. Hughes
Agilent Technologies Company, Pleasanton, CA

Abstract

A rapid, simple, and sensitive electrospray LC/MS method has been developed for the analysis of LSD, iso-LSD, and 2-oxo-3-hydroxy-LSD using the HP 1100 LC/MSD system. Calibration curves are linear from 25–5000 pg/ml with correlation coefficients greater than 0.99. Quality-control results obtained from this LC/MS method show good precision and accuracy and are in agreement with results obtained using an independently validated GC/MS/MS method. This LC/MS method has been used to quantitate LSD and 2-oxo-3-hydroxy-LSD and to detect iso-LSD in urine samples from LSD users. Most of the urine samples tested by this LC/MS method have shown much higher concentrations of the metabolite 2-oxo-3-hydroxy-LSD than of LSD. Therefore detection and quantitation of the metabolite extends the detection time-window.

Introduction

Lysergic acid diethylamide (LSD) is one of the most difficult drugs of abuse to detect in urine because the parent drug is excreted at very low concentrations. Several methods for confirmation of LSD in urine have employed GC/MS or GC/MS/MS, both of which require derivatization of the analyte. The use of LC/MS for the analysis of LSD and related compounds does not require derivatization of the analytes, thus simplifying the procedure. Methodology that utilizes tandem mass spectrometry (MS/MS) often improves the specificity and signal-to-noise ratio, both of which can be important when trace concentrations of drugs in biological tissues are being measured. However, MS/MS instrumentation is prohibitively expensive for most toxicology laboratories. We report a relatively rapid and sensitive analytical method for the quantitation of LSD, iso-LSD, and 2-oxo-3-hydroxy-LSD in urine using a single-quadrupole LC/MS system.

Experimental

The system included an Agilent 1100 Series binary pump, vacuum degasser, autosampler, thermostatted column compartment, diode-array detector, and an LC/MSD. The LC/MSD was used with the electrospray ionization (ESI) source. Complete system control and data evaluation were carried out using the Agilent ChemStation for LC/MS.

Sample Preparation and Extraction

Drug-free urine was fortified with known concentrations of LSD, iso-LSD, and 2-oxo-3-hydroxy-LSD for preparation of standard curves. Control samples were fortified with known concentrations of LSD and 2-oxo-3-hydroxy-LSD prepared from separate lots. Urine (4 ml) spiked with internal standards (2-oxo-3-hydroxy-LAMPA and LSD-d_3) was subjected to solid-phase extraction. Bond-Elut Certify columns were used according to the manufacturer's procedure for basic drug extraction. The final sample residue was reconstituted in 50 µl (20 µl injected) of LC mobile phase for analysis by LC/MS.

Chromatographic Conditions	
Column:	Metasil Basic 3 µm, 3 × 100 mm (Metachem)
Mobile phase:	A = 0.1% formic acid in water B = methanol
Gradient:	start with 15% B at 1 min 15% B at 2 min 30% B at 6 min 30% B
Flow rate:	0.5 ml/min
Column temp:	25°C
Injection vol:	20 µl
Diode-array detector:	signal 210, 10 nm reference 360, 10 nm
MS Conditions	
Source:	ESI
Ionization mode:	positive
Vcap:	2000 V
Nebulizer:	45 psig
Drying gas flow:	13 l/min
Drying gas temp:	350°C
SIM ions:	m/z 356.4 (2-oxo-3-hydroxy-LSD and 2-oxo-3-hydroxy—LAMPA, 324.4 (LSD and iso-LSD), 327.4 (LSD-d_3)
Peak width:	0.12 min
Time filter:	on
Fragmentor:	70 V

Results and Discussion

A recently identified metabolite of LSD, 2-oxo-3-hydroxy-LSD, is generally present in the urine of LSD users at higher concentrations than LSD. Although iso-LSD is not a metabolite of LSD, it is often detected in the urine of LSD users because of its presence as a contaminant in the LSD sold on the street. Figure 1 shows the extracted ion chromatograms (EICs) for blank urine fortified with the internal standards. Figure 2 shows the EICs for an extracted urine standard fortified with each analyte at 50 pg/ml. Calibration curves for each analyte were linear from 25 to 5000 pg/ml with correlation coefficients (r^2) greater than 0.99 (see Figure 3).

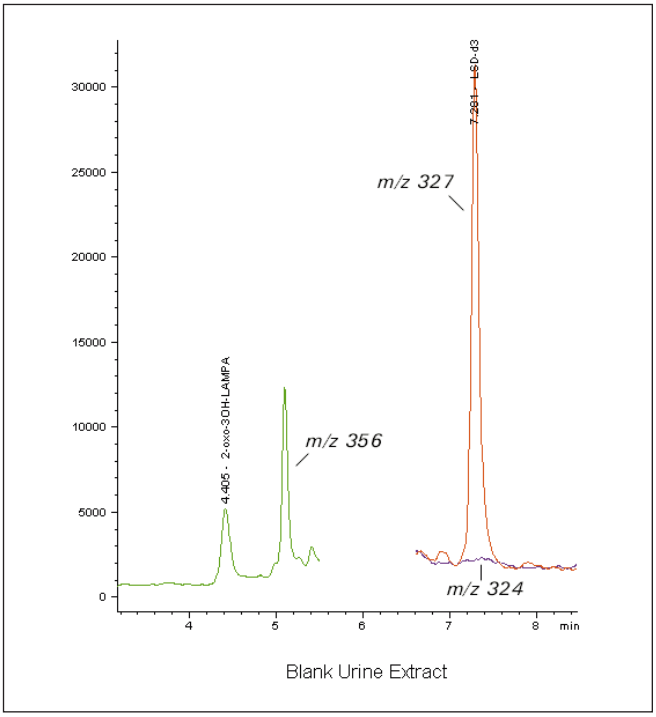

Figure 1. Extracted ion chromatograms of blank urine extract.

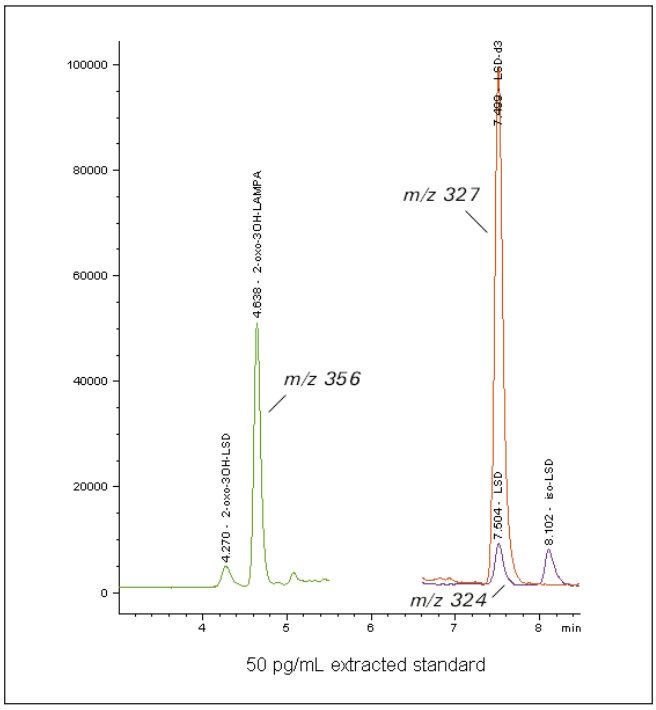

Figure 2. Extracted ion chromatograms of fortified urine extract (50 pg/ml).

Quality control samples fortified with 250 pg/ml of LSD quantitated within 12% of the target concentration, with a coefficient of variation of 2.3% (see Table 1).

Results from this LC/MS method compared favorably to those obtained by Northwest Toxicology (Salt Lake City, Utah) using an independently validated GC/MS/MS method in which a triple-quadrupole MS system and TMS derivatives were used for GC/MS/MS of these analytes. Control urine was fortified at 40, 400, and 4000 pg/ml with both LSD and 2-oxo-3-hydroxy-LSD, and analyzed using both the LC/MS method and the GC/MS/MS method. Results agreed within 11% at the 40-pg/ml level (one-fifth of the 200 pg/ml positive cut-off), and within 6% and 5% at the 400 and 4000-pg/ml, respectively (see Table 2).

Table 1. Intra-assay precision.

Sample	LSD (pg/ml)
250 a	212
250 b	217
250 c	218
250 d	219
250 e	226
mean	219
Std error of mean	2.27
% of target	87.6
% CV	2.03

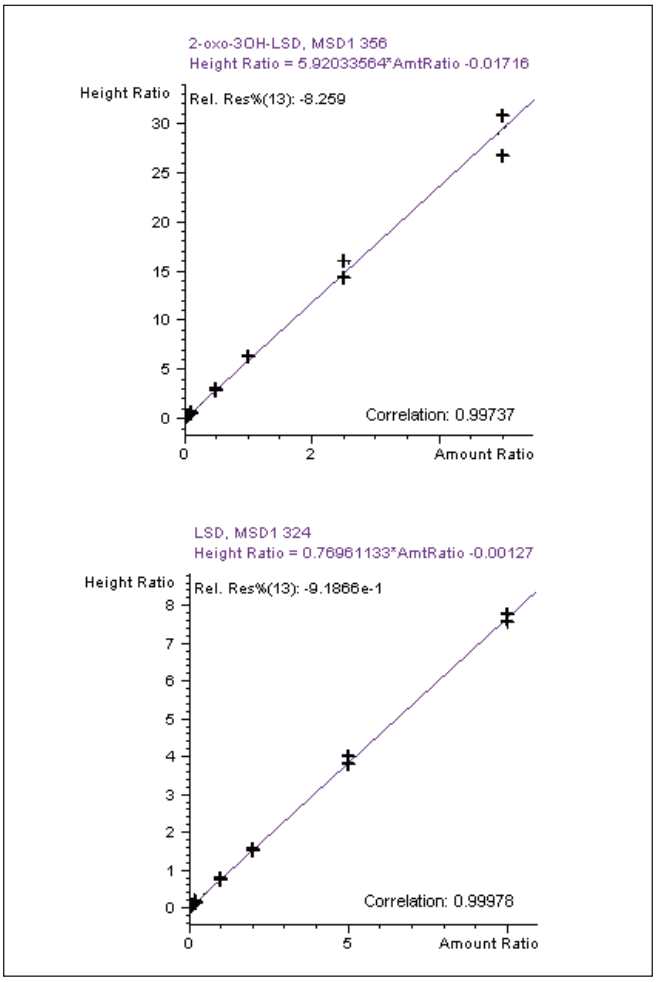

Figure 3. Calibration curves for LSD and 2-oxo-3-hydroxy-LSD.

Table 2. Comparison of LC/MS and GC/MS/MS results.

Concentrations in pg/ml			
Analyte, (target conc, pg/ml)	**LC/MS***	**GC/MS/MS***	**% Difference**
LSD (40)	38.5 ± 0.8	43.4 ± 1.0	11.3
LSD (400)	399.6 ± 1.1	418 ± 2.1	4.4
LSD (4000)	4170.4 ± 34.3	3918 ± 43.5	6.4
2-oxo-3-OH-LSD (40)	37.1 ± 0.2	41.9 ± 0.7	11.4
2-oxo-3-OH-LSD (400)	376.4 ± 5.1	366 ± 6.4	2.8
2-oxo-3-OH-LSD (4000)	3752.8 ± 106.4	3963 ± 61.7	5.3

*Plus or minus standard error of the mean; n = 3.

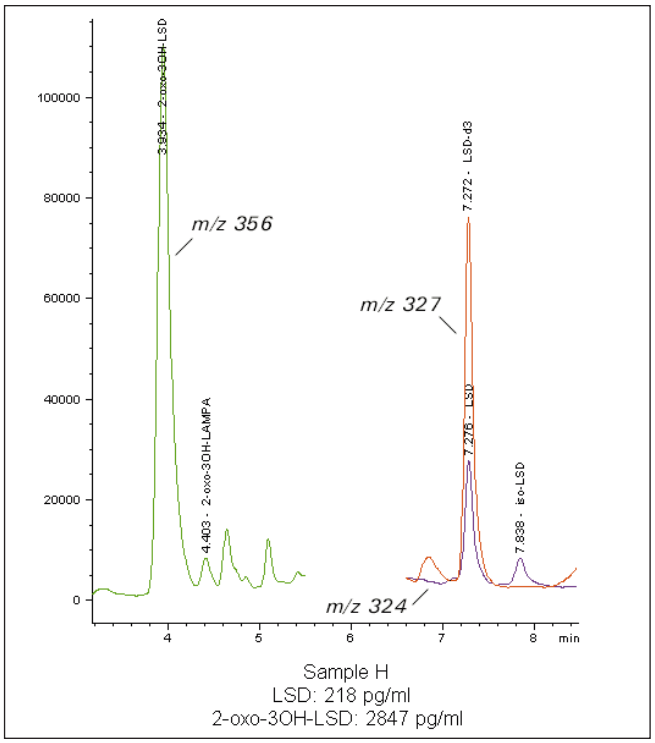

Figure 4. Extracted ion chromatograms for extract of positive urine sample H.

Urine samples (n = 10) that had been previously screened positive for LSD by RIA were analyzed using the LC/MS method (see Table 3). The table summarizes the LSD and 2-oxo-3-hydroxy-LSD concentrations in these samples. Using the LC/MS method, LSD concentrations for positive samples ranged from 57 to 1197 pg/ml. Concentrations of 2-oxo-3-hydroxy-LSD for positive samples ranged from 95 to 3470 pg/ml. Figure 4 shows the extracted ion chromatograms for sample H, for which the metabolite concentration was more than 10 times the concentration of the parent drug.

A rapid, simple, and sensitive method for analyzing LSD, iso-LSD, and 2-oxo-3-hydroxy-LSD in urine has been developed for use with a single-quadrupole LC/MS system. This method produces results that compare favorably to those obtained with a triple-quadrupole GC/MS/MS system.

Table 3. Urine concentrations from LSD users.

Sample ID	LSD, pg/ml	2-Oxo-3-OH-LSD OH-LSD, pg/ml
A	960	192
B	nd	654
C	1092	254
D	527	3470
E	1197	1646
F	209	402
G	57	95
H	218	2847
I	343	3435
J	165	2382

nd = not detected

Acknowledgments

The authors would like to thank Shaundel Percey and Scott Reuschel of Northwest Toxicology for the preparation and analysis of quality-control samples by GC/MS/MS.

Dr. Matt Slawson is a research toxicologist, ***Dr. Carol O'Neal*** is a research associate, and ***Dr. Rodger Foltz*** is Associate Director at the Center for Human Toxicology. ***Dr. Thomas Kupiec*** is currently Director of Laboratories at Analytical Research Laboratories, Edmond, Oklahoma. ***Dr. John Hughes*** is an applications consultant at the Agilent Technologies Company.

**Printed in the U.S.A. April 2000
(23) 5968-4920E**